Remarks

Claims 76, 79-82, 90, 91, 99, 100, 141, and 142 are pending. Claim 76 is proposed to be amended herein. The proposed amendment finds support throughout the specification and particularly at e.g., paragraphs [0064], [0068], and [0194]. New claims 143 and 144 are proposed to be added herein. New claim 143 finds support at e.g., paragraphs [0082] and [0194], while new claim 144 finds support at e.g., Example 7 particularly paragraph [0146], lines 5-10.

Rejections under 35 USC 112, first paragraph

Claims 76, 79-82, 90, 91, 99, 100, 141, and 142 are rejected for lacking enablement under 35 U.S.C. 112, first paragraph. The Office Action argues that the specification is not enabled for the breadth of the claim beyond inhibiting PSA expression using a 600nt expression cassette in human rhabdomyosarcoma cells. Applicant respectfully disagrees, for the following reasons.

An important aspect of the presently claimed invention pertains to reducing the interferon response normally observed upon transfection of a dsRNA effector molecule into a cell by expressing the dsRNA effector molecule intracellularly. To support the notion that intracellular expression of a dsRNA reduces the interferon response compared to extracellular dsRNA delivery, Applicants submit herewith a declaration under 37 C.F.R. § 1.132 from Dr. C. Pachuk, an expert in the field of dsRNA mediated gene silencing and an inventor on the present application. In the declaration, Dr. Pachuk states the following:

> "From my experience in the field of dsRNA mediated gene silencing, it is clear that when dsRNA molecules are administered intracellularly (e.g., by microinjection, electroporation, or expression from a vector) there is generally little to no stress response observed, while the same dsRNA molecules administered extracellularly (e.g., by liposome-mediated transfection) can induce a much stronger stress response. Intracellular administration or expression of dsRNA molecules can prevent a dsRNA molecule from interacting with cell surface Toll like receptors (TLRs) to activate a cellular immune response. In addition, extracellular administration of dsRNA molecules can result in the uptake of dsRNA molecules into endosomes." [Pachuk declaration; Paragraph 5]

Intracellular expression of a dsRNA molecule in a cell to reduce the interferon response was not known in the art at the time of filing and is exemplified in the present specification in Examples 10 and 11.

Applicants previously submitted further evidence in post-filing literature by Robbins et al., *Nature Biotechnology* 24(5):566-571 (2006), entitled "Stable expression of shRNAs in human CD34+ progenitor cells can avoid induction of interferon responses to siRNAs in vitro." The main thrust of this post-filing publication is that expression of shRNAs in cells can avoid the induction of interferon responses to siRNAs. That is, while exogenously delivered siRNAs induced an interferon response, the same sequences when expressed in the cell did not stimulate such a response. Thus, the Robbins et al. reference confirms the results of the presently claimed invention and the statements provided by Dr. Pachuk in the accompanying declaration.

With regard to the Robbins et al. reference, the Office Action states that it is "clear that the stress response is unpredictable and that the expression vector used did not avoid the stress response, contrary to what Applicants appear to be suggesting based on Robbins." The Office Action cites Kenworthy et al., and Bauer et al. to highlight the alleged unpredictability of the interferon response.

Far from highlighting unpredictability, the Kenworthy et al. reference states "The majority of the shRNAs that we use in the lab do not activate RIG-I expression and IFN signaling despite having essentially the same structure as sh-B971," (see e.g., page 6591, column 2, lines 1-3), "IFN activation *depended on sequence*," (see e.g., page 6588, column 1, lines 29-33, emphasis added), and "So far as we know, ours is the *first report* of IFN activation in the target cells by shRNAs delivered by lentiviral transduction" (see e.g., page 6597, column 1, lines 15-17, emphasis added). Kenworthy et al. clearly indicates that the sh-B971 shRNA does not behave in the same manner as the majority of shRNAs expressed intracellularly; that is, it induces a stress response *when the majority of other shRNAs expressed intracellularly do not*. Thus, Kenworthy et al. supports the conclusion that it is **rare,** at best, for a dsRNA effector, e.g., an shRNA, to elicit an appreciable interferon response when expressed in the cell. While Applicants in no way acknowledge that inhibition of target expression by intracellular expression of a dsRNA is unpredictable, the law does not require 100% predictability for enablement. The MPEP acknowledges as much, e.g., at §2164.03, which states " but even in unpredictable arts, a disclosure of every operable species is not required" to meet the enablement requirement.

The disclosure by Kenworthy et al. is cited by the Examiner to support the Office Action's position regarding the alleged unpredictability of using dsRNA effector molecules without causing an interferon response. However, taken as a whole, the Kenworthy et al. reference indicates that the

induction of the interferon response is sequence-specific and does not occur with all intracellularly expressed shRNAs, and simply does not support the argument of the Office Action that the use of intracellularly expressed dsRNA effector molecules is unpredictable. Instead, it is clear from the disclosure of Kenworthy et al. that induction of an appreciable interferon response to dsRNA expressed in the cell is an unusual phenomenon and was related to a particular shRNA (B971). That is, only a very small number of intracellularly expressed shRNAs appreciably induce an interferon response, and according to Kenworthy et al., "a majority" of intracellularly expressed shRNAs do not induce an interferon response. Applicants submit that an event that occurs, or in this instance, does not occur "a majority" of the time is predictable.

The statements of Kenworthy et al. that only some dsRNAs elicit a stress response are confirmed by Dr. Pachuk in the accompanying declaration, which states:

> "My experience in the field of dsRNA mediated gene silencing supports the statements of Kenworthy et al. outlined above, in that only in rare cases does an intracellularly expressed dsRNA provoke a significant stress response. Expression of a dsRNA molecule in the cell very predictably reduces the level of stress response compared to that induced by administering the same dsRNA extracellularly. While some dsRNAs may indeed still cause a minor stress response when administered intracellularly, the level of the stress response is, in every instance of which I am aware, reduced compared to the stress response observed when that dsRNA is administered extracellularly. The discovery that one could reduce the level of an immune response by expressing a dsRNA molecule inside the cell, rather than administering it to the outside of the cell, was an important discovery that helped to further the field of dsRNA- mediated gene silencing." [Pachuk declaration; Paragraph 7]

Applicants note that the claims as amended do not require the absence of a stress response, but rather the level of any such response, e.g., the level of apoptosis is decreased "by at least 20% compared to the level of apoptosis in a population of cells transfected with the same double stranded RNA produced outside the cell." This is fully consistent with Dr. Pachuk's statement that

> "Expression of a dsRNA molecule in the cell very predictably reduces the level of stress response compared to that induced by administering the same dsRNA extracellularly. While some dsRNAs may indeed still cause a minor stress response when administered intracellularly, the level of the stress response is, in every instance of which I am aware, reduced compared to the stress response observed when that dsRNA is administered extracellularly." [Pachuk declaration; Paragraph 7]

The Office Action also points to the Bauer et al. reference to support its conclusion that the claimed invention is not enabled. The Office Action specifically points to language at the end of the Introduction to the Bauer et al. paper as indicating that "certain features .... such as the shRNA or modification of the passenger strand, as done by Bauer" have not been identified and somehow are required for the claimed invention. The specific language of the Bauer et al. reference is as follows:

> "We show here that the induction of the interferon-response gene Oas1 by expression of first generation shRNA can be abolished by the introduction of the targeting sequence into a miR-30 backbone, whereby the modification of the passenger strand seems to be a crucial feature to avoid innate cellular immune response."

First, Applicants submit that the Kenworthy et al. reference relied upon by the Office Action directly contradicts a conclusion based on Bauer et al. that only the approach taken by Bauer et al. can work. That is, Kenworthy et al. did not use the miR-30 backbone or specific approaches for modification of the passenger strand, yet still achieved target gene inhibition without stress response induction for the majority of shRNAs tested. Robbins et al. also supports the fact that one need not necessarily take the approach taught by Bauer et al. to achieve target gene inhibition without appreciable stress response induction. It is clear from both the Robbins et al. and the Kenworthy et al. references that approaches other than the use of the miR-30 backbone for shRNA expression *can and do work without strongly invoking the innate immune response*. Applicants submit that the possibility that there may be ways to improve upon a method does not negate the patentability of that method by lack of enablement – if this were the case, few, *if any*, methods or processes would be patentable.

The declaration by Dr. C. Pachuk further supports that the methods of Bauer et al. are not the only methods useful in dsRNA mediated gene silencing, and points out a deficiency in Bauer et al. with regard to assessing the stress response. The declaration states the following:

> "I note in particular that the Bauer et al. investigators did not measure the level of stress response when the shRNA is introduced from outside the cell, and consequently could not compare the level of stress response to that produced by the same shRNA expressed intracellularly. The results of Bauer et al. with the particular constructs they used cannot support a conclusion that the expression of their constructs does not result in reduced stress response relative to the stress response that would occur if the same shRNAs were introduced from outside the cell. While I acknowledge that it would

> require experimental data to absolutely confirm, based on my considerable experience in precisely this area, I would expect that the shRNA used in the Bauer et al. reference would induce a greater stress response when administered extracellularly than when expressed in the cell. While Bauer et al. may have found a method to further improve (i.e., decrease) the level of stress response induction when dsRNAs are expressed intracellularly, this result has no relevance to the invention as presently claimed." [Pachuk declaration, Paragraph 9]

Further, Applicants note that the statement in the Bauer et al. reference relied upon by the Office Action is equivocal. The authors state, for example, that "the modification of the passenger strand *seems to be* a crucial feature to avoid innate cellular immune response." To suggest, on the basis of one author's statement of what "seems to be crucial" that the *only* approach to avoiding the innate immune response is to use the specific miR-30 backbone flies in the face of evidence published in other references that clearly demonstrate other approaches that work to inhibit target gene expression without appreciably inducing the innate immune response.

As noted above, Applicants propose herein to amend the claims to include the limitation "decreases the level of apoptosis by at least 20% compared to the level of apoptosis in a population of cells transfected with the same double stranded RNA produced outside the cell." Under this language, a degree of stress response is permitted, so long as the dsRNA effector molecule specifically downregulates target gene expression and shows a reduction in apoptotic death of at least 20% when the effector is expressed in the cell as compared to cells treated with the same dsRNA extracellularly. Thus, as is clear from Robbins et al. and also from Kenworthy et al., the majority of intracellularly expressed dsRNA effector molecules will fall within these parameters. As proposed to be amended, those rare dsRNAs that do induce an appreciable stress response fall outside the scope of the claims. Applicants submit that the teachings of Kenworthy et al. and Robbins et al. confirm that most shRNAs can be successfully employed using intracellular expression as required in the methods of the presently claimed invention.

Further with regard to enablement, the Office Action states:

> "At the time the instant application was filed, and even to date, nucleic acid based therapies were highly unpredictable. The field of RNA interference was in its infancy and gene specific dsRNA inhibition in mammalian cells was also highly

> unpredictable, even in cells in culture and the ability to inhibit gene expression was variable and unpredictable among different cell lines and different target genes,"

The Office Action continues, stating that the claimed invention:

> "would result in an undue burden upon those of ordinary skill in the art beyond the downregulation of PSA expression in human rhabdomyosarcoma cells using the 600nt expression cassette."

Applicants respectfully disagree. There are certain properties relating to dsRNA inhibition of gene expression that are sequence dependent and others that essentially are not. It is known in the art that not all dsRNA effector sequences are ideal for inhibiting gene expression because the nucleic acid sequence can affect factors like RNA folding, and other factors can play a role in determining which dsRNA effector molecules are preferred over other dsRNA effector molecules. Nevertheless, while the particular effector sequence may not be known, essentially *any* gene can be targeted for down-regulation via some group of dsRNA effector molecules. The approach of designing and testing a number of dsRNA effector sequences to identify those that will work is routinely undertaken in both academic and industry settings. The law expressly permits experimentation that is not undue - see, e.g., *PPG Indus., Inc. v. Guardian Indus. Corp.,* 75 F.3d 1558, 1564 (Fed. Cir. 1996), *Atlas Powder Co. v. E.I. duPont de Nemours & Co.,* 750 F.2d 1569, 1576 (Fed. Cir. 1984) and *W.L. Gore & Assoc., Inc. v. Garlock,* 721 F.2d 1540 (Fed. Cir. 1983). While the Office Action argues that the literature indicates that siRNA methods are unpredictable, the fact is that methods to identify dsRNA effector sequences that work have been, and are used routinely by those of skill in the art in a number of contexts and therefore do not present an undue burden. Further, by recognizing that intracellular expression of dsRNAs avoids strong induction of the innate cellular immune response to dsRNA, which is one of the reasons dsRNA approaches can fail, the inventors of the claimed invention have actually improved the predictability of downregulating target genes via dsRNA.

The declaration by Dr. C. Pachuk provides the following conclusion:

> "In view of the above, it is my opinion that neither Bauer et al. nor Kenworthy et al. supports the conclusion regarding unpredictability drawn by the Examiner. In particular, even where there may remain some degree of stress response to dsRNAs

expressed in the cell, in my opinion, based on considerable experience in this area, the level of that stress response will generally be considerably less than the response to the same dsRNA administered extracellularly." [Pachuk declaration; Paragraph 10]

In view of the above, Applicants respectfully submit that the claims as proposed to be amended are fully enabled under 35 U.S.C. §112, First Paragraph. Applicants respectfully request reconsideration and withdrawal of the enablement rejection.

Conclusion

In view of the amendments and remarks provided above, all issues raised in the Office Action are addressed herein. Reconsideration of the claims is respectfully requested.

Should any other fees be associated with this submission, the Applicants hereby authorize the Commissioner to charge such fees to Nixon Peabody Deposit Account No. 50-0850. Any overpayments should also be credited to said Deposit Account.

Respectfully submitted,

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